Title: Cloning RFP into BBa_F2620
Goal: Insert RBS/RFP/terminator behind LuxR/Lux promoter
Date range: July 10 – August 7
Members that worked on project: Hannah Young, Josephina Hendrix

Summary: The goal of this project was to insert the RFP gene (BBa_J04450 without its promoter, but with ribosome binding site intact) into BBa_F2620 (LuxR gene followed by Lux promoter) on a 1C3 backbone.
New part number: BBa_K1718003
Part size: 1793 bp

DNA Source

- BBa_F2620 was resuspended from 2015 Distribution Kit Plate 3 well 4O.

Overnight:

LuxR:

- 1 overnight
  - 5 ml LB
  - 25 ul Chloramphenicol (34 ug/ml)

Mini-prep:

- LuxR mini-prep concentration: 241.6 ng/ul

PCR:

- PCR of: BBa_J04450
- Purpose: To isolate the RBS (B0034), RFP gene (E1010), and terminator (B0015) from the LacI (R0010) promoter and the backbone. Linearized DNA to be used later in a restriction digest.
- PCR polymerase: Q5

Reagent	25 ul reaction	Concentration
10 uM F. Primer	1.25 ul	0.5 uM
10 uM R. Primer	1.25 ul	0.5 uM
DNA (89.9 ng/ul)	3 ul	267.9 ng total, 10.72 ng/uL
Q5 High-Fidelity 2X Master Mix	12.5 ul	1 x
Water	7 ul	

Reaction conditions:

Step	Temp (C)	Time
I. Denature	98	30 s

35 cycles	98	10 s
	71	20 s
	72	25 s
Final Extension	72	2 min

- Primers used: GEM027: J04450F, GEM027: J04450R
- Annealing temp: 71 degrees Celsius
- Extension time: 25 sec extension

PCR cleanup:
- Elution volume: 30ul
- Concentration: 241.6 ng

Digestion of RBS/RFP/terminator (BBa_B0034, BBa_E1010, BBa_ B0015)
- Purpose: Gel Extraction

Digestion of: LuxR (BBa_F2620)
- Purpose: Gel Extraction
- Enzymes Used:

Reagent	50 ul reaction	Concentration
Cutsmart Buffer	5 ul	1 x
SpeI-HF	1 ul	10 units
PstI-HF	1 ul	10 units
LuxR DNA (241.6 ng/ul)	2.5 ul	604 ng total, 12.08 ng/ul
Water	40.5 ul	

Gel:
- Latter: 2-log
- Gel concentration: 1% agarose

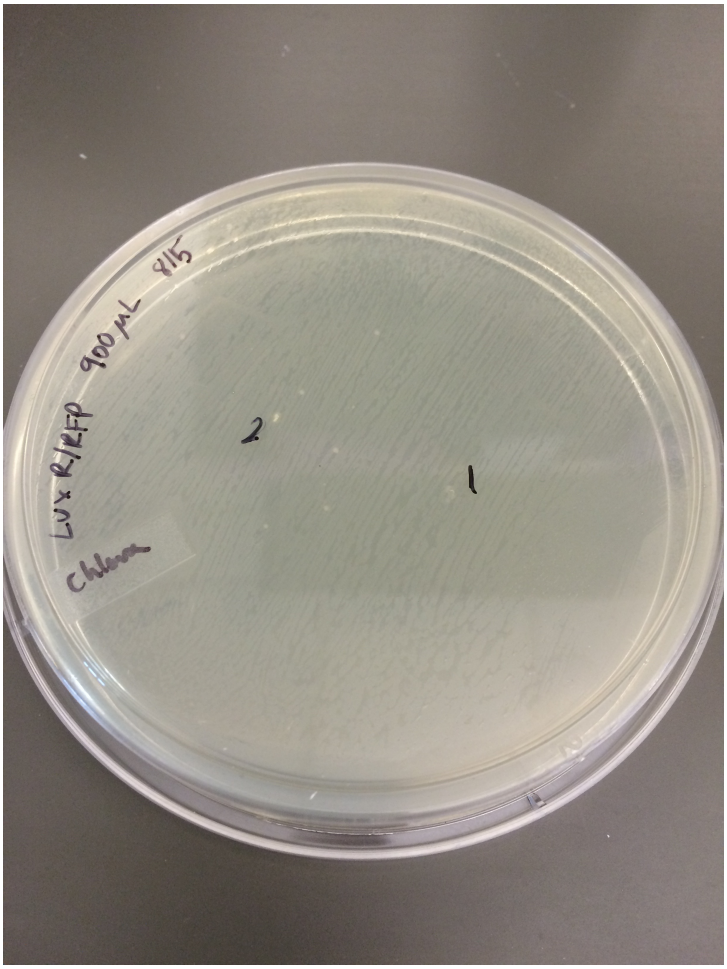
Gel extraction:
- Extracted: LuxR cassette

RFP gel extract for ligation obtained from Josephina Hendrix

Ligation:
- Ligation of LuxR gel extract digested with SpeI and PstI and RFP gel extract digested with XbaI and Pst1

Reagent	20 ul reaction
T4 Ligase Buffer	2 ul
T4 Ligase	1 ul
LuxR DNA (gel extracted)	3.05 ul
RFP DNA (gel extracted)	2.54 ul
Water	11.41 ul

Transform ligation results:
100ul: no colonies
900ul:



minus insert: no colonies
negative control: no colonies

Digestion of: RFP/LuxR Ligation product, Colony #1

- Purpose: Check for insert size

Reagent	10 ul reaction	Concentration
Cutsmart Buffer	1 ul	1 x
EcoRI-HF	0.2 ul	2 units
PstI-HF	0.2 ul	2 units
DNA (181.8 ng/ul)	1 ul	181.8 ng total, 18.18 ng/ul
Water	7.6 ul	

Digestion of: RFP/LuxR Ligation product, Colony #2

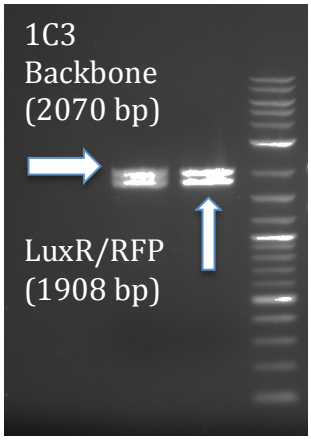
- Purpose: Check for insert size

Reagent	10 ul reaction	Concentration
Cutsmart Buffer	1 ul	1 x
EcoRI-HF	0.2 ul	2 units
PstI-HF	0.2 ul	2 units
DNA (202.4 ng/ul)	1 ul	202.4 ng total, 20.24 ng/ul
Water	7.6 ul	

2 Overnights of ligation product, one from each colony.
Minipreps of 2 ligation products.
Digest of products with EcoRI and PstI to run on a gel.

Gel:

- Latter: 2-log
- Gel concentration: 1% agarose
- Expected: 2 bands
    - LuxR/RFP Insert (1908 bp)
    - 1C3 Backbone (2070 bp)



Lane 1: LuxR/RFP Colony 1
Lane 2: LuxR/RFP Colony 2
Lane 3: 2 log ladder

Sequence:

- Date sent: 8/13/15
- Primers used: VF2 sequencing primer, VR sequencing primer, RFPseq2 primer
- Result: 0 mismatches

Part submission:

- New part name: N-acyl-homoserine-lactone inducible LuxPr promoter generating RFP